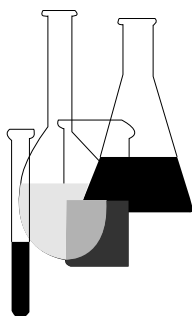




Ecological Effects Test Guidelines

OPPTS 850.2450 Terrestrial (Soil-Core) Microcosm Test



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading “Environmental Test Methods and Guidelines” or in paper by contacting the OPP Public Docket at (703) 305-5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

Final Guideline Release: This guideline is available from the U.S. Government Printing Office, Washington, DC 20402 on *The Federal Bulletin Board*. By modem dial 202-512-1387, telnet and ftp: fedbbs.access.gpo.gov (IP 162.140.64.19), or call 202-512-0135 for disks or paper copies. This guideline is also available electronically in ASCII and PDF (portable document format) from the EPA Public Access Gopher (gopher.epa.gov) under the heading “Environmental Test Methods and Guidelines.”

OPPTS 850.2450 Terrestrial (soil-core) microcosm test.

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is the OPPT guideline under 40 CFR 797.3775 Soil-Core Microcosm Test (proposed in the FEDERAL REGISTER of September 28, 1957 (52 FR 36363)).

(b) **Purpose.** This guideline is intended for use in developing data on the toxicity and fate of chemical substances and mixtures (“test substances”) subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94–406, 96 Stat. 2083, 14 U.S.C. 2801 *et seq.*). This guideline prescribes tests using soil-core microcosms to provide information on the potential fate and ecological effects of chemical substances released to a specific terrestrial ecosystem. The United States Environmental Protection Agency (EPA) will use data from these tests in assessing the hazard of a test substance to the environment.

(c) **Definitions.** The definitions in section 3 of TSCA and Part 792—Good Laboratory Practice Standards of this chapter, apply to this test guideline. The following definitions also apply:

Bioconcentration factor (BCF) means the ratio of the concentration of test substance in plant tissue (i.e., biota) to that in soil.

Biota means the organisms in the soil at the time of extraction of the core and the natural vegetation or crop species introduced as the autotrophic component. Biota includes all heterotrophic and carnivorous invertebrates in the soil and all soil and plant bacteria, fungi, and viruses.

Carrier means the organic solvent, solubilizer and/or other substance used to disperse the test substance into microcosm water.

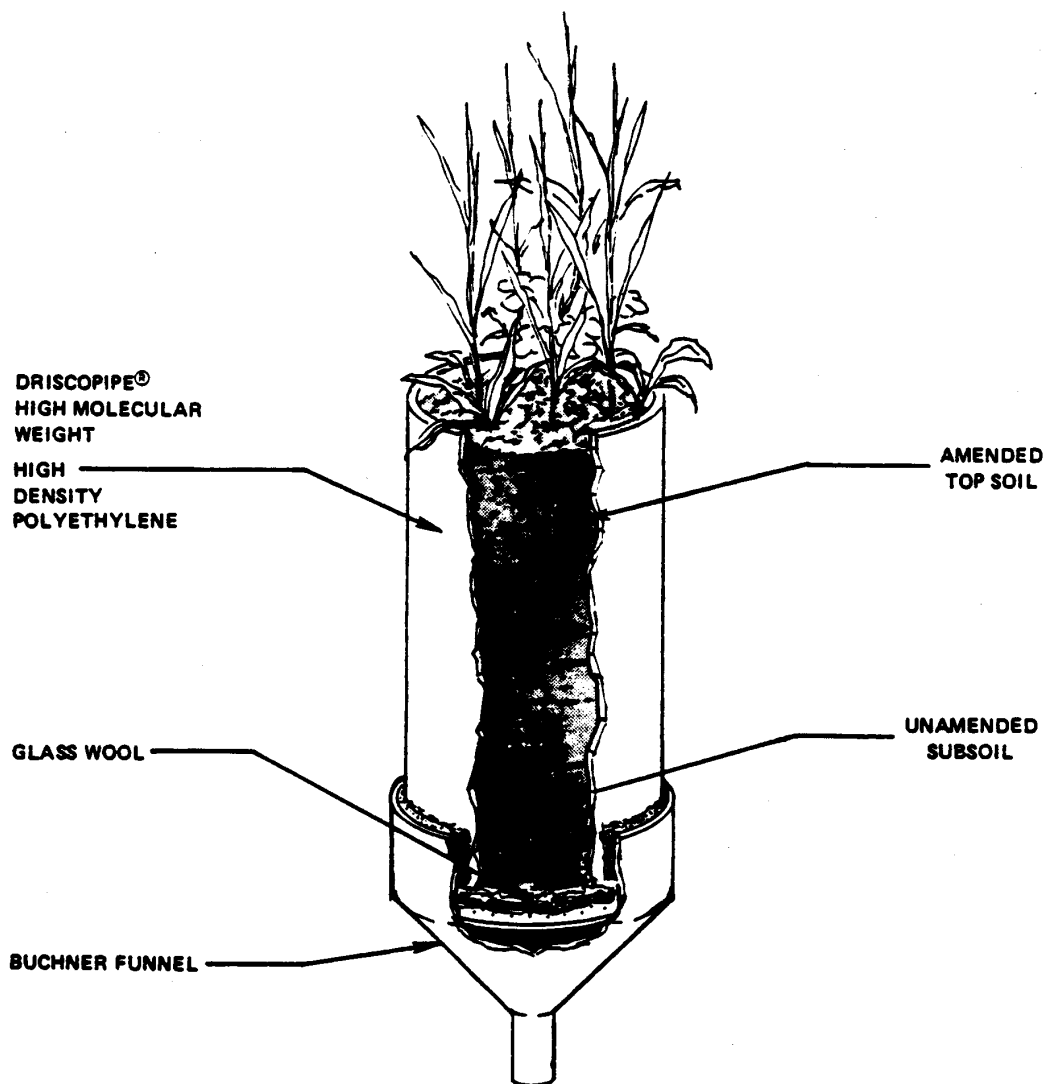
Soil-core means an intact, undisturbed (nonhomogenized) core that is extracted in situ from a soil type typical of the region or site of interest and that is of sufficient depth to allow a full growing season for the natural vegetation or the crops selected, without causing the plants to become significantly root bound.

Soil-core microcosm means a physical miniaturized model of an interacting community of autotrophs, omnivores, herbivores, carnivores, and decomposers within an intact soil profile.

(d) **Test procedures**—(1) **Summary of the test.** The purpose of the soil-core microcosm test is to determine the potential fate and ecological effects of a chemical substance, including its transformation products, released to a specific terrestrial ecosystem. A soil core, as shown in the

following Figure 1, containing biota typical of the region of interest, is treated with the test substance under controlled conditions in either a growth chamber or greenhouse. The test is usually continued for a minimum of 12 weeks from first application of the test substance to final harvest. Single or multiple applications of the test substance may be chosen, depending on the expected mode of introduction of the test substance into the environment. Leachate, soil, and plant samples are analyzed to evaluate the environmental fate of the test substance. Ecological effects of the substances are evaluated on the basis of measurements of primary productivity and nutrient loss, as well as on determinations of BCFs and observations of plant condition.

Figure 1.—Microcosm Structure and Materials



(2) **Application of the test substance.** (i) Whenever possible, the test substance should be radiolabeled. The label may be ^{14}C , stable iso-

topes such as ^{15}N , or other suitable labels and, if possible, should be located in a portion (or portions) of the molecule known or expected to persist and/or have biological activity. For single chemical substances, two or more portions of the molecule may need to be labeled; in the case of mixtures, each component must be labeled and studied separately.

(ii) The test substance should be applied in a form which is reasonably consistent with the form in which it is expected to be released into the environment. The method and pattern of application should also reflect the actual or predicted field situation.

(A) If the primary mode of exposure to the test substance is anticipated to be by addition of pH-adjusted, reverse osmosis (RO) water or rainwater containing appropriate concentrations of the substance, the following procedure is recommended.

(1) Test substances which are likely to be released into the environment as a liquid or powder, and which can be mixed with water, should be applied as a single dose of liquid in a volume sufficient to bring the A horizon of the soil surface of the microcosm to field capacity.

(2) Water simulating rainfall or leaching should be filtered rainwater from the site being evaluated or purified (i.e., reverse osmosis) untreated laboratory water with a known chemical composition.

(3) The volume of reverse osmosis (RO) water or rainwater required for laboratory microcosms may be determined on-site using a microcosm of the same soil type without vegetation. The volume selected should be identical for all microcosms and be sufficient to bring the A horizon of the soil surface to field capacity.

(4) Carriers other than water should not be used unless they are likely to be released into the environment with the test substance. If a carrier is necessary, acetone or ethanol should be considered; however, the use of carriers should be avoided unless they are essential to produce a realistic exposure.

(B) Several typical methods of application are suggested for particular types of test substances:

(1) If the test substance is likely to be a contaminant of irrigation water, it should be applied periodically, such as daily or weekly, in proportionate concentrations, such that the total amount applied equals the desired level of treatment.

(2) If the test substance does not mix with water, it should be applied as evenly as possible to the top of the microcosm. If the microcosm is simulating an agricultural system, the test substance should be mixed into the topsoil before planting.

(3) If the test substance is normally sprayed on growing plants, the desired amount should be mixed with the volume of water necessary to wet the soil surface and wet the plants to the point at which they begin to drip. A chromatography sprayer should be used to spray plants that are past the seedling stage. The manufacturer's recommendations for field spraying the test substance should be followed as closely as possible, and the test should be terminated at least 8 weeks after plants are sprayed.

(C) Water solubility and dissociation constant(s) of the test substance and soil pH must be considered in determining the formulation of the test substance. The treatment concentrations should bracket the known or expected environmental concentration of the test substance. However, if the environmental concentration is unknown and cannot be estimated, the maximum concentration of the substance in solution should not exceed half of saturation.

(3) **Range-finding test.** (i) A range-finding test may be conducted if definitive testing is necessary and to determine the concentrations of test substance to be used in the definitive test.

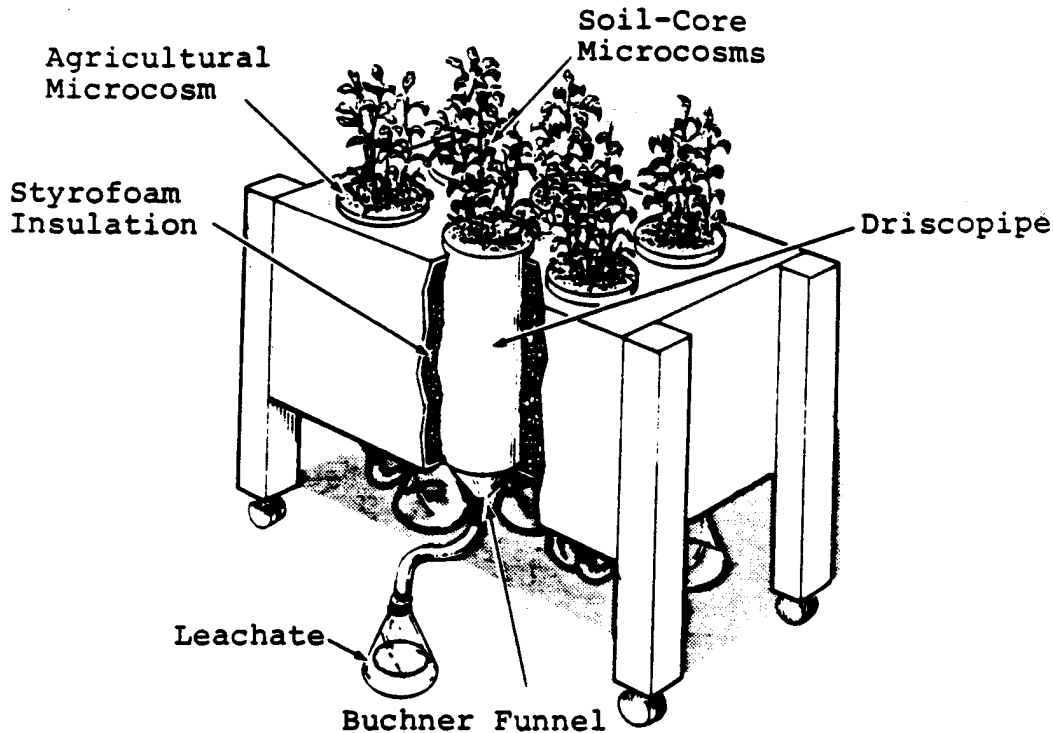
(ii) Physicochemical information supplied for the test substance should be used to tailor the general range-finding test procedures to the specific substance.

(iii) Phytotoxicity and/or bacteriostatic action, if known, should be considered in selecting exposure concentrations for the range-finding test. Only one concentration greater than that known to cause at least a 50 percent change in plant growth or a 50 percent change in bacterial growth/respiration should be tested. Also, the lowest concentration to be used should not be lower than a factor of 10 times the analytical detection limit in soil and 100 times the analytical detection limit in water.

(iv) The range-finding test should last at least 4 weeks from first application of the test substance to plant harvest. At the beginning of the test, microcosms should be treated with a minimum of five concentrations of the test substance. Three replicate microcosms are used for each concentration and for the control, resulting in a total of at least 18 microcosms. Concentrations typically used for treatment are 0.1, 1.0, 10, and 100 mg/L (ppm), if actual environmental concentrations are not known and cannot be predicted. If appropriate, 1,000 µg of test substance per gram of top 15 cm of dry soil should also be used. The bulk density (g/cm³) of the dry soil should be used to calculate the exposures. Depending on the expected mode of release of the test substance, each recommended concentration may be applied as a single dose or may be divided into multiple doses. In either case, the total amount of test substance applied for any given concentration must be the same.

(v) Each microcosm cart, in the following Figure 2, holding one replicate of each test substance concentration and a control, should be moved once per 7 days in the greenhouse to minimize location-induced effects.

Figure 2.—Arrangement of Microcosms in Styrofoam Cart



(vi) Losses of calcium, potassium, nitrate-nitrogen, orthophosphate, ammonium-nitrogen, and dissolved organic carbon (DOC) should be measured in soil leachates. Leachate should be collected in acid-washed, 500-mL flasks attached to the end of the Buchner funnel by inert plastic tubing. Leaching is induced by adding a volume of rainwater or RO water above that necessary to bring the soil profile to field capacity. The volume of additional water needed to induce leaching in a specific core should be determined when the cores are extracted from the field. The volume of rainwater or RO water needed should be recorded. Flasks to collect the leachate may be supported by a wooden board fastened under the microcosm cart. The volume of leachate should be recorded and the pH determined using a glass electrode. Samples should be centrifuged at low speed (e.g., 5,000 rpm) and filtered through a 0.45- μ m filter. The sample should be divided into two aliquots and stored in the dark at 4 °C with blanks consisting of distilled water and reference standards in quantities sufficient for instrument calibration.

(vii) At the termination of the range-finding test, soil samples should be collected from the top, middle, and bottom of the 60-cm soil cores. If the radiolabeled test substance or its transformation products are not detected in the deeper soil samples by liquid scintillation counting, soil

samples at the end of the definitive test should be taken nearer the top of the soil column.

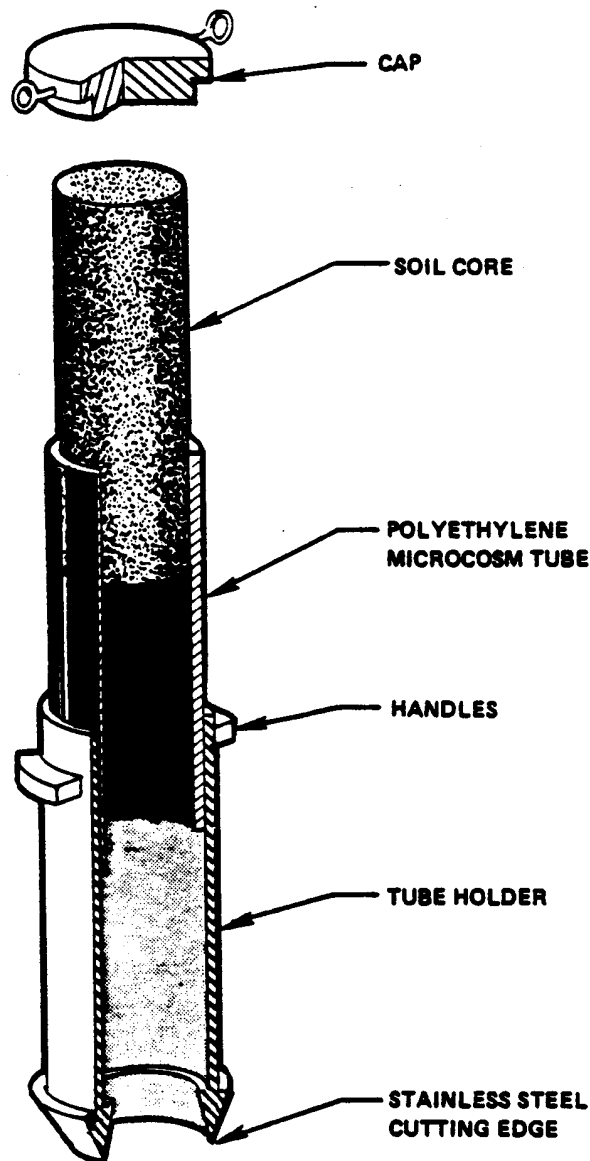
(viii) If no discernible effects of the test substance are detected during the range-finding test at one-half of saturation or 1,000 $\mu\text{g/g}$ (whichever is higher), including visible effects of plant injury, no definitive test is necessary.

(4) **Definitive test.** (i) The purpose of the definitive test is to determine the potential fate and ecological effects of a test substance, including its transformation products, in a site-specific natural grassland or agricultural ecosystem.

(ii) Chemical substances with high vapor pressures or high Henry's law constants should not be tested in the soil-core microcosm as prescribed in this guideline.

(iii) Soil cores (17-cm diameter by 60-cm deep) should be extracted from either a natural grassland ecosystem or a typical agricultural soil in the region of interest. The intact system should be extracted with a specially designed, steel extraction tube, as shown in the following Figure 3, and a backhoe. Disturbances during extraction and preparation of the soil core should be minimized.

Figure 3.—Diagram of Microcosm Extraction Tube



(iv)(A) For an agricultural microcosm, the soil which is plowed (generally, the top 15 cm of soil) should be moved aside and saved. Once the core is cut by the leading edge of the driving tube, it should be forced up into the microcosm tube or Driscopipe as demonstrated in Figure 1 in paragraph (d)(1) of this guideline. The Driscopipe should then contain a 45-cm core of subsoil. The homogenized topsoil that was saved should be backfilled into the upper 15 cm of the microcosm tube after it has been returned to the laboratory.

(B) A mixture of grasses and broad leaves (e.g., legumes) should be included in the agricultural microcosm. Seeds from two or three species of grasses or legumes that are typically grown together as an agricultural crop in the region of interest should be chosen and planted. The rate of

seed application should duplicate standard farming practice for the region of interest. Seeds should be planted evenly and covered to an appropriate depth with soil.

(v)(A) For a natural grassland microcosm, the vegetation covering the natural grassland ecosystem should be clipped to a uniform height before the core is extracted. Natural plant cover should be sufficiently diverse to be representative of plant species in the region of interest.

(B) The soil core from the grassland ecosystem should be removed as a single unit (soil and Driscopipe) from the extraction tube, taken to the laboratory, and placed on a Buchner funnel covered by a thin layer of glass wool. The funnel and tube should be washed with acid (50 percent concentrated HNO_3) before use and then rinsed with RO water.

(vi) Six microcosms are typically contained in a moveable cart which is packed with styrofoam beads, as shown in Figure 2 in paragraph (d)(3)(v) of this guideline.

(vii) An appropriate random process should be used, such as completely randomized, randomized block, or Latin-square design, to assign microcosms to different concentrations of the test substance.

(viii) The method and pattern of application and the form of the test substance used should approximate a reasonable scenario of how the substance is expected to be released at the site in question (see paragraph (d)(2)(ii) of this guideline).

(ix) At the beginning of the test, microcosms should be treated with at least three concentrations of the test substance. Ten replicate microcosms should be used for each of the three concentrations and for the control, for a total of 40 microcosms. The treatment concentrations should be selected to produce a 20 to 25 percent change in plant productivity in each treatment based on results from the range-finding test. The 10 replicate microcosms in each treatment group should be used as five replicate pairs.

(x) Microcosms that have been paired for analysis should be placed in different carts to ensure that environmental conditions are as uniform as possible.

(xi) The structure, materials, and treatment of control microcosms should be the same as that of exposed microcosms except that none of the test substance is applied.

(xii) Microcosms should be watered as dictated by a predetermined water regime based on site history with either reverse osmosis (RO) water or with rainwater that has been collected from the region of interest, filtered, and stored in a cooler at 4 °C. Care should be taken to provide sufficient water for normal plant functions without overwatering. If the

test substance is applied as an aerosol or powder, plants should be sprinkled immediately after treatment to avoid resuspension of particulates and reduce the potential for cross-contamination of exposure concentrations.

(xiii) Data regarding solubility of the test substance in water and its capacity to sorb to soils should be used, along with the results of the range-finding test, to help determine the appropriate regime for soil leachate collection and analysis. Microcosms should be leached, as described in paragraph (d)(3)(vi) of this guideline, at least twice before application of the test substance and once every 2 or 3 weeks after such application. The frequency of rainfall in the region of interest should be considered when a leaching regime is selected. Water to leach the microcosm should be added to each microcosm over an 8- to 12-h period to avoid waterlogging the soil surface. To ensure that all test microcosms will leach within a 2-day period, at least 15 percent more soil cores should be extracted than are required for the tests. When the microcosms are leached before planting, those which do not leach, leach too quickly, or take longer than 2 days to produce 100 mL of leachate after the soil has been brought to field capacity should be discarded.

(xiv) Light intensity measurements should be taken daily, but should be taken at least at the beginning and end of the test.

(xv) Temperatures should be monitored continuously at the top of the plant canopy.

(xvi) Plants should be carefully monitored for changes in physical appearance, such as stunting, discoloration, or chlorosis and/or necrosis of the leaves.

(xvii) To measure plant primary productivity, plants from the natural grassland or agricultural microcosm should be harvested at the end of the test period (a minimum of 12 weeks) and, possibly, once or twice during that period, depending on the types of plants growth. For example, vigorously growing grasses may be sampled during the middle of the test. Plants should be clipped to approximately 2.5 cm above the soil surface. Harvested plants should be stored in separate paper bags for each microcosm, and air-dried, oven-dried, or both soon after harvest. The test may be extended beyond 12 weeks to accommodate plant species which take longer to reach the desired maturity (e.g., seed production). Plant productivity, depending on the plant species, may be measured as total yield and/or yield by plant part, e.g., total biomass or grain. Minimally, plant productivity should be measured as oven-dry weight expressed as grams per square meter; in the grassland microcosms, monocotyledons and dicotyledons should be separated for both plant productivity measurements and radiochemical assay.

(xviii) Nutrient losses should be sampled in soil leachates. Nutrients to be measured should be selected based on the properties of the test sub-

stance and the results of the rangefinding test (see paragraph (d)(3)(vi) of this guideline).

(xix) Samples of soil leachate, plant tissue (including roots and shoots), and soil from three depths should be analyzed for radioactivity, and identification and quantification of the test substance. The three soil depths should be selected based on soil sorption of the test substance and results from the range-finding test. These depths should be relatively close to the soil surface (1 to 2 cm) for radiolabeled chemicals that are strongly sorbed to soils. If any isotope appears in the leachate during the range-finding test, the depth selection should be lower in the soil profile. The entire soil layer should be taken as the sample, and then subsamples should be homogenized and extracted with solvents appropriate for the test substance. Additional extraction steps, such as acidification and extraction with non-polar solvents, Soxhlet extractions with polar and/or nonpolar solvents, alkaline or acid hydrolysis with or without heat, detergent extractions, or protease digestion may be necessary. The ^{14}C in the soil or plant samples which cannot be extracted should be oxidized and analyzed as $^{14}\text{CO}_2$ and reported as bound residue. Extracts and the oxidized or dissolved samples should be counted by ^{14}C liquid scintillation.

(xx) Soil invertebrates and microbes may be sampled at the end of the test.

(5) **Analytical measurements**—(i) **Chemical.** (A) Standard analytical methods, if available, should be used to establish actual concentrations of solutions of the test substance and should be validated before beginning the test. An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, cause positive or negative interference. The pH of these test solutions should also be measured before use.

(B) The fate or final distribution of the test substance and its transformation products should be determined by methods appropriate to the test, including sensitivity factors adequate to verify exposure and distinguish between the test substance, its transformation products, and naturally occurring materials present in the test system. Whenever possible, this should involve use of a radiolabeled test substance, and subsequent analysis of the primary microcosm compartments and soil leachate for radioactivity and chemical identity.

(C) Identification and quantification of the test substance or its transformation products, expressed as a percent of the original application, in various compartments of the microcosm should be performed using gas-liquid chromatography (GLC), thinlayer chromatography (TLC) or high-pressure liquid chromatography (HPLC). TLC autoradiography using no-screen X-ray film for chromatographed fractions which are found to be radioactive by liquid scintillation counting may be most cost-effective.

However, whenever possible, the identity of the test substance and its transformation products in fractions which are found to be radioactive by liquid scintillation counting should be verified by GLC, HPLC, or other appropriate methods. Also, the concentration of the test substance and transformation products should be verified by an alternative chromatographic method (e.g., HPLC or GLC) with known standards.

(D) Standard techniques suitable for nutrient analysis may include atomic absorption spectrophotometry for calcium and potassium, and a Technicon Autoanalyzer II for nitrate nitrogen, orthophosphate, DOC, and ammonium nitrogen.

(ii) **Numerical**—(A) **Experimental design.** Analysis of variance (ANOVA) calculations should be performed to test for position effects within the carts and within the environmental area where the test is performed. If these tests are significant at the 5-percent level ($P < 0.05$), this should be accounted for in subsequent statistical analyses.

(B) **Productivity.** (1) The effects of different concentrations of the test substance on productivity can be evaluated initially by using side-by-side histograms displaying calculated means (expressed as grams per square meter), variances, 95-percent confidence intervals, and two standard errors for air- and oven-dried biomass collected from control and treatment groups. Early evaluation will indicate whether logarithmic or some other transformation of the data is necessary for graphic display and analysis. Pair-wise comparisons may be necessary for variables which were measured only once during the 12-week test.

(2) Biomass data should be analyzed by ANOVA and least significant differences multiple-range procedures. The level of significance for all tests should be at the 5-percent level. Where treatment effects and interactions between and among various factors are important, a two-way ANOVA or factorial analysis should be performed.

(3) Regression/correlation analysis should be performed on plant productivity results. Obvious recording or reporting errors in the data should be excluded but noted in the final report. If substantial data are excluded, deficiencies in quality control may necessitate repeating the test. Once outlying values have been detected and removed from further statistical evaluations, regression models or probit analysis should be used to estimate the concentration at which 50 percent of the productivity observed in controls occurred in the treated groups (EC50). Ordinary linear-least-squares-regression analysis should initially be performed, and predicted responses in each group should be compared using a Student t-test (one-sided). If productivity appears to be bimodal when compared to controls, a two-sided Student t-test may be necessary. It may also be necessary to transform the data or fit a quadratic or cubic least-squares-regression model to the data for this type of response. Positional effects should be included

in the data. Computer software packages such as SAS (Statistical Analysis System) or BMCP (Biomedical Computer Program) may be useful.

(C) **Plant injury.** Statistical analyses of the effects of the test substance and transformation products on the appearance of plants are not necessary unless there is a clearly identifiable pattern of effects. If deemed necessary, types of injury should be ranked by severity. A non-parametric test, such as the Kruskal-Wallis test, should then be performed.

(D) **Nutrient losses.** (1) Based on the nutrients selected for analysis in soil leachate, the total cumulative loss of each nutrient from each microcosm should be calculated by multiplying the concentration of the nutrient collected at each sampling time by the total volume leached from that microcosm for that collection date and adding the product to the previous sum of total loss.

(2) Means (\pm SE) of the cumulative nutrient losses for each treatment concentration for each collection date should be plotted as a function of days after seeding for the agricultural microcosm or days after application of the test substance for the natural grassland microcosm. Zero loss should be the starting point. If there was no leachate for any microcosm during a particular collection period, the data point should be recorded as zero so that no data are considered missing.

(3) A one-way ANOVA should be performed on total cumulative nutrient loss data at the end of the test, to evaluate effects of different concentrations of the test substance. A multiple-range procedure, such as Duncan's, should be used to determine which specific treatment means are different from each other.

(4) Regression and/or correlation analysis comparing losses of each nutrient analyzed versus plant productivity should be performed.

(E) **Chemical fate analysis**—(1) At the end of the test, the mass balance or final distribution of the test substance and its transformation products in above- and below-ground plant tissues, selected depths through the soil profile, and losses through soil leaching and gaseous transport should be calculated for each concentration of the substance tested.

(2) Calculations should be based on measured radioactivity in a specific compartment of the microcosm, on a per-gram basis, times the total weight or volume of test substance in that compartment, expressed as dry weight when appropriate. All calculations should be corrected for radioactive decay (as appropriate) that has occurred since the beginning of the test. Quantities of the test substance and its transformation products should be expressed as a percent of the original application of the test substance.

(3) Statistical analyses should be performed for each exposure concentration on any differences in distribution of the test substance in the

primary compartments of the microcosm and in soil leachate. Multicompartmental modeling and multivariate analysis of variance may also prove useful in assessing the fate of a test substance and its transformation products.

(4) The time to reach steady-state loss through leaching and the time to initiate leaching should be calculated for each exposure concentration.

(F) **Radioactivity budget.** Calculation of a complete mass balance of all radioactivity should be performed as follows:

(1) Total radioactivity added per microcosm should be calculated based on the decay rate of the radioactive label (e.g., ^{14}C), the total amount of radioactive label added to the test substance initially, the length of time between formulation and microcosm exposure (radioactive decay), and the particular concentration of the test substance added to the microcosm.

(2) Total radioactivity removed from the microcosm should be calculated based on the following data:

(i) Soil leachate concentration times the volume of soil leachate lost per collection date.

(ii) Calculated gaseous losses of the test substance.

(iii) The type of radiolabel and rate of radioactive decay of that label during the test.

(3) Total radioactivity remaining in the microcosm can be calculated based on analysis of the radioactivity in each of the following primary compartments:

(i) Above-ground plant tissues.

(ii) Below-ground plant tissues, i.e., cleaned of soil particles.

(iii) The soil profile.

(G) **Bioconcentration.** The ratio of the amount of radioactivity in above-ground plant tissues to the amount in the top 15 cm of soil should be calculated on a concentration-perunit, dry-weight basis. Side-by-side histograms of the BCFs should be compared for statistical differences.

(H) **Soil organisms.** Appropriate statistical methods should be used to evaluate the distribution and abundance of soil invertebrates and function of the soil microbial community with respect to treatment concentrations.

(e) **Test conditions—(1) Test species—(i) Selection.** Biota should be included in the microcosm. A mixture of two or three species of grasses or broad leaves, such as legumes, representative of the area or region

where the test substance is expected to be released or applied to crops or soil, should be included in the agricultural microcosm. Chosen species should be compatible and able to grow to maturity in the limited surface area of the microcosm. Thus, large crops such as corn or sorghum cannot be used under these guidelines.

(ii) **Seed selection.** Information on seed lot, seed year, or growing season collected and germination percentage should be provided by the source of the seed. Only untreated seed (not treated with fungicides, repellants, etc.) taken from the same lot and year or season of collection should be used in a given test. In addition, all seed of a species used in a test should be of the same size class, and that size class which contains the most seed should be selected and used in a given test. Any damaged seed should be discarded.

(2) **Facilities**—(i) **Apparatus.** (A) The greenhouse or growth chamber should provide adequate environmental controls to meet light and temperature specifications.

(B) Laboratory facilities for test substance determinations should include: Nonporous floor covering; absorbent bench covering with nonporous backing; and adequate disposal facilities to accommodate radiolabeled test solutions and wash solutions containing the test substance at the end of each test, and any bench covering, laboratory clothing, or other contaminated materials, appropriate equipment for analytical determinations, drying ovens, refrigerators, and standard laboratory glassware.

(C) A specially designed steel extraction tube and a backhoe are needed to extract soil cores.

(ii) **Containers and supporting equipment.** (A) For the definitive test, at least 18 microcosms are required. The three basic materials used for a single microcosm are: A 60-cm long Driscopipe tube (17.5 cm diameter), a 186 mm-diameter porcelain Buchner funnel, and a thin layer of glass wool (see Figure 1 in paragraph (d)(1) of this guideline). Containers used in each test should be of equal size and volume and possess the same configuration.

(B) Three mobile carts should be used to hold 18 microcosms. The carts should be designed to hold adequate styrofoam beads for insulation in Figure 2 under paragraph (d)(3)(v) of this guideline.

(iii) **Cleaning.** All equipment used in the test should be cleaned before use and should be washed according to good standard laboratory practices, to remove any residues remaining from manufacture or use. A dichromate solution should not be used for cleaning containers. Disposal of all detergents and acids that have been used to clean the Driscopipe funnels, and laboratory glassware, and disposal of all liquid and solid sam-

ples and remaining undisturbed portions of the test system should conform to applicable existing Federal regulations.

(3) **Test parameters.** Microcosms should be kept in a greenhouse or environmental chamber with controlled environmental conditions.

(i) The temperature should approximate outdoor temperatures that occur during a typical growing season in the region of interest.

(ii) The photoperiod and intensity of light typical for the growing season in the region of interest should be simulated. Light for the test system can be supplied by artificial lighting suitable for plant growth in either an environmental chamber or greenhouse or can be the natural photoperiod occurring in a greenhouse. If the test is performed in an environmental chamber, the daily photoperiod for the microcosm should be at least the average monthly incident radiation (quantity and duration) for the month in which the test is being performed, with a cycle equivalent to the natural photoperiod.

(f) **Reporting.** The report should include, but not necessarily be limited to, the following information:

(1) Name and address of the facility performing the study and the dates on which the study was initiated and was completed, terminated, or discontinued.

(2) Objectives and procedures stated in the approved protocol, including any changes in the original protocol.

(3) Statistical methods used for analyzing the data.

(4) The test substance identified by name, Chemical Abstracts Service (CAS) registry number or code number, source, lot or batch number, strength, purity, and composition or other appropriate characteristics, such as water solubility and vapor pressure at 25 °C.

(5) Stability of the test substance and, if used, control substances under the conditions of administration.

(6) A description of the methods used, including:

(i) Greenhouse or environmental chamber conditions, including type and size, temperature, photoperiod, and light intensity.

(ii) Source, any special treatment, and chemical composition of the water used.

(iii) Method and equipment used to extract the soil core.

(iv) Randomization procedures used to position microcosms and assign test concentrations to particular microcosms.

(v) Frequency, duration, and methods of observations.

(7) A description of the test system used including:

(i) The soil core, including chemical, biological, and physical characteristics, source, soil type, and when applicable, identification of plant species included in the natural vegetation or the scientific names and sources of the agricultural plants selected and histories of the species, e.g., percentage of seeds germinating and seed size class.

(ii) Planting procedures and any special handling of seed before planting.

(iii) Number of total weight (for smaller species) of seeds tested per concentration (in agricultural microcosm).

(8) A description of the experimental design, test substance concentrations, method and pattern of application, replicates, controls, and carriers. The reported results should include:

(i) Results of the range-finding test and measurements.

(ii) Results of the definitive test including:

(A) Visible effects of the test substance on intact plants.

(B) Total productivity and/or yield by plant part (e.g., total biomass or grain) expressed as grams per square meter oven-dry weight.

(C) Losses of selected nutrients in leachates.

(D) Percent distribution of the test substance and its transformation products in the primary compartments of the microcosm, including above- and below-ground plant tissues and selected depths through the soil profile expressed as dry weight, and in soil leachate expressed as grams per square meter. Losses via gaseous transport should be estimated and expressed as milligrams per cubic meter.

(E) A radioactivity budget including total radioactivity added to, removed from (via soil leaching, gaseous transport, and radioactive decay), and remaining in each microcosm (plant tops and roots and selected soil depths).

(F) Bioconcentration of the test substance in above-ground plant tissue expressed as the ratio of the concentration in plant tissue to the concentration in the top 15 cm of dry soil.

(9) A description of all circumstances that may have affected the quality or integrity of the data.

(10) The name of the sponsor, study director, principal investigator, names of other scientists or professionals, and the names of all supervisory personnel involved in the study.

(11) A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis. Results of the analysis of data should include the concentration response curves with 95-percent confidence limits, the results of a goodness-of-fit test, e.g., X^2 test, and EC50s.

(12) The signed and dated reports of each of the individual scientists or other professional involved in the study including each person who, at the request or direction of the testing facility or sponsor, conducted an analysis or evaluation of data or specimens from the study after data generation was completed.

(13) The location where all specimens, raw data, and the final report are stored.

(14) The statement prepared and signed by the quality assurance unit.